Work plan and Methodology

A. Method development experiment plan:

- Extensive literature review will be done prior to initiate any practical work.
- Procurement of pure drug, impurities, column and dosage form.
- Information of solubility profile, analytical profile and stability profile of analyte.
- Gathering the information about the structures of the molecule and the impurities likely to be present /possible.
- Based on above possible impurity structure polarity will be decided.
- Choose the mode of the chromatography reverse phase or normal phase.
- Selection of column and the bonding phase :

  ←---------Non-polar--------moderately polar----------Polar----------→

  C18 < C8 < C6/C4 < Phenyl < Amino < Cyano < Silica
- **Selection of mobile phase**: Initially mobile will be selected which content phosphate buffer and based on elution pattern organic modifier will be used for optimization of mobile phase.
- **Selection of Detector**: PDA (photodiode Array) Detector will be selected if the chromophores present in the compounds. If the compounds are not having chromophores, will choose other detectors like RI, ELSD.
- **Ion-pair reagents:** Ion pair reagents can be used when necessary, when a closely related compounds separation is required.
- **Selection of a diluent:** in which main analyte and impurities are soluble and based on peak shape and recovery of peak of interest it will be optimized.
- Select an initial **flow rate** of 1.0mL/min or 1.5ml/min and select column **temperature** as Ambient (25–30°C) and based resolution of peak it will be optimized.
- **Sample** to be used for development: Mixture of all impurities will be used for method development to establish the separations.
- **Forced degradation** studies or Stress testing studies: This study will be performed to provide foundation for developing a suitable stability indicating method.

B. Method Validation plan:

Based on above development and optimization of method, developed method will be validated for its intended use as per ICH guideline and as below mention parameter will be covered

1. System Suitability
2. Specificity (Placebo Interference)
3. Method precision.
4. Ruggedness
5. Limit of detection & quantification
6. Linearity.
7. Accuracy.
8. Range.
9. Robustness (Variations).
10. Specificity (Forced degradation / Stress study).